

WHAT IS CLAIMED IS:

1. A method of establishing a clonal embryonic stem cell line capable of sustaining a phenotype of normal embryonic stem cells following at least eight months of *in vitro* culturing, the method comprising culturing an individual embryonic stem cell for at least eight months in a serum-free medium, thereby establishing the clonal embryonic stem cell line capable of sustaining said phenotype of normal embryonic stem cells following at least eight months of *in vitro* culture.

2. The method of claim 1, wherein said individual embryonic stem cell is a human embryonic stem cell.

3. The method of claim 1, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of 28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.

4. The method of claim 3, wherein said non-increasing population doubling time is selected from a range of 33 to 37 hours.

- (d) dissociating said inner cell mass-derived cell mass into dissociated cells;
- (e) culturing said dissociated cells on mouse embryonic feeder fibroblasts, thereby generating dissociated cell-derived colonies;
- (f) selectively harvesting from among said dissociated cell-derived colonies a colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli; and
- (g) dissociating said colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli into individual cells thereby obtaining said individual embryonic stem cell.

10. The method of claim 1, wherein said serum-free medium includes feeder fibroblasts.

11. The method of claim 10, wherein said feeder fibroblasts are murine.

12. The method of claim 10, wherein said feeder fibroblasts are embryonic.

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13. The method of claim 1, wherein said serum-free medium includes 0.4 to 40 ng/ml bFGF.

14. The method of claim 1, wherein said serum-free medium includes 1 to 16 ng/ml bFGF.

15. The method of claim 1, wherein said serum-free medium includes 2 to 8 ng/ml bFGF.

16. The method of claim 1, wherein said serum-free medium includes 4 ng/ml bFGF.

17. A clonal human embryonic stem cell line being capable of sustaining a normal embryonic stem cell phenotype following at least eight months of *in vitro* culturing.

18. The clonal human embryonic stem cell line of claim 17, wherein said *in vitro* culturing is effected on mouse embryonic feeder fibroblasts in serum-free medium supplemented with basic fibroblast growth factor.

19. The clonal human embryonic stem cell line of claim 17, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of

28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.

20. The clonal human embryonic stem cell line of claim 17, wherein said non-increasing population doubling time is selected from a range of 33 to 37 hours.

21. The clonal human embryonic stem cell line of claim 17, wherein said non-decreasing telomere length is selected from a range of 4 to 16 kb.

22. The clonal human embryonic stem cell line of claim 17, wherein said non-decreasing telomere length is selected from a range of 8 to 12 kb.

23. The clonal human embryonic stem cell line of claim 17, wherein said pluripotentiality is characterized by the capacity to differentiate into endodermal, mesodermal and ectodermal cells.

24. A clonal human embryonic stem cell line being capable of sustaining a normal embryonic stem cell phenotype following at least twelve months of *in vitro* culturing.